Title: Charting Taxa in *Amanita* Section *Amidella* (*Basidiomycota* : *Amanitaceae*)

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Abstract

Species determination in the *Amidella* clade is notoriously difficult, because of the relative dearth of field characters and the rather common occurrence of homoplasies. This results in a substantial number of misnamed and unnamed collections, a misapprehension of the geographic range of known species, and a gross underestimation of the number of species it contains. To assess the diversity that should be considered as part of *Amidella*, DNA sequences available for this group were retrieved in public nucleotide databases, using a combination of approaches to achieve a comprehensive representation. Phylogenetic analysis based on the aligned ITS sequences, consistently with the results from other molecular markers (nLSU, RPB2, TEF1, BTUB), suggests five major clades: one containing the type species *Amanita volvata*, another for *Amanita ponderosa* and allies, a third one (roughly half of all species) with *Amanita lepiotoides*, and two others without valid species yet. Consensus rRNA folding structures were obtained for these clades, maintaining their congruence and separation from neighbouring congeneric clades. At species level, around 78 clades were delimited, of which only up to 17 can be assigned a valid name, with a few more corresponding to provisional taxa listed in the amanitaceae.org website. Two further species without assigned sequences might correspond to the proposed clades. The current evidence suggests a rather narrow geographic range for most of these clades. This study provides a phylogeny-arranged outlook of the worldwide distribution of *Amidella* species, and a framework for designing clade-specific molecular markers to assist in identification.

Keywords
*Amanita*, *Amidella*, taxonomy, molecular phylogeny, biogeography, RNA consensus folding.

## Introduction

The creation of *Amidella* as a taxonomic group dates from Edouard-Jean Gilbert's proposal, within family *Amanitaceae* by the same author, initially as a separate genus and subsequently as a subgenus in *Amanita* Pers. (Neville & Poumarat 2004). Both the name and the classification scheme that has endured since then underscore the importance attached to the basidiospore amyloid reaction with iodine which the name refers to (Konrad and Maublanc 1948).

The current taxonomic arrangement within *Amanita* recognises *Amidella* at the section level in subgenus *Amanitina*, bracketed by sections *Roanokenses* and *Arenariae* (Cui et al. 2018). This is the culmination of a process whereby several groups with amyloid basidiospores segregated from the original concept of *Amidella* (Wolfe et al. 2012; Cui et al. 2018; Riccioni et al. 2019), leaving the name *Amidella* solely for a morphologically distinct, relatively small group of species (Cui et al. 2018; Tulloss and Yang 2024).

In contrast to its demarcation from other congeneric groups, making it fairly easy to identify specimens as 'Amidella', their identification at species level has remained very challenging (Tulloss and Yang 2024). It may be achieved at regional level, as done recently for Europe with prominent recourse to ecological characteristics such as soil, vegetation and phenology (Arraiano-Castilho et al. 2022). This notwithstanding, too many specimens have been assigned equivocally with species epithets such as *volvata*, *avellaneosquamosa* or *curtipes*. The problems arising from this are very difficult to tackle, and the only compelling argument against misidentifications have been the evident phylogenetic disparities (Moreno et al. 2008; Kim et al. 2013; Cho et al. 2015; Cui et al. 2018).

The ingrained notion of *Amidella* as a small group of species, in spite of some evidence against it (Cui et al. 2018; Tulloss and Yang 2024), favours the continued misapplication of known epithets and does not encourage the exploration of any meaningful taxonomic subdivisions. Partial phylogenetic sampling does little to address these issues, not to mention mere blast searches; a comprehensive overview of the diversity of *Amidella* is definitely needed.

This study attempts such comprehensiveness based on publicly available molecular databases. The approach cannot cover a few known species lacking molecular representation, but it provides a novel picture of the group, recognising more than 70 clades at species level, most of them undescribed, and demonstrating a phylogenetic substructure of great value for separating sympatric species. Knowledge gaps, such as the need for new morphological criteria and the underrepresentation of vast geographical regions, are clearly defined under the new taxonomic view provided here.

# Materials and Methods

## Adopted identifications

There are concerns about the correct application of some names. Following known authorities of genus *Amanita*, a tentative set of sequenced specimens are adopted as reference (Table 1).

## Sources and search strategies

### Named sequences in Amanitaceae.org (Tulloss and Yang 2024)

The GenBank accession numbers for all sequences in Section *Amidella* listed in the details tab of each species were collected and used to retrieve from GenBank the full FastA records using the NCBI Batch Entrez interface (https://www.ncbi.nlm.nih.gov/sites/batchentrez last accessed June 30, 2024).

### 5.8S phylogeny

On February 25th 2023 a total of 7,045 sequences under the genus name *Amanita*, containing at least one of the nuclear ribosomal internal transcribed spacers (ITS), were downloaded from the GenBank database, and processed by ITSx (Bengtsson-Palme et al. 2013) in the PlutoF platform (Abarenkov et al. 2010), producing a total of 6,445 5.8S sequences that were aligned in UGene (Okonechnikov et al. 2012) and further processed with MEGA (Tamura et al. 2021), using the Maximum Likelihood tree construction method (see Outline of phylogenetic analysis, below). The resulting dendrogram was then searched for species epithets known to belong to Section *Amidella*. Three major clades were identified (Table 2), all containing many accessions without known epithets, and the GenBank accessions contained in them were collected, and used to retrieve from GenBank the full FastA records using the NCBI Batch Entrez interface.

A preliminary phylogenetic analysis of the retrieved ITS sequences was made after this, to constitute an outgroup made only from sequences that branched out closer to the known *Amidella* sequences. Those that ended up in the outgroup were all from clade I (Table 2), all from Section *Roanokenses*.

### Affinity with collected sequences

Two strategies were followed: first, using the voucher identifiers, an in-house wrapper script was developed to parse the output of esearch | efetch | xtract from Entrez Direct (Kans 2013) and used to retrieve the corresponding non-ITS records from GenBank (i.e. for the nuclear ribosomal 28S large subunit gene LSU, for the second largest protein subunit of the RNA polymerase II RPB2, for the translation elongation factor EF-1 alpha TEF1, and for the tubulin beta chain BTUB) that matched the specimens already available; second, using the BLAST homology algorithm, either in the PlutoF platform for the ITS sequences (marking for the retrieval of environmental sequences), or in the NCBI interface for LSU, RPB2, TEF1 and BTUB.

### Additional sequences

Several other sequences were spotted as they were referred to in specimen observation online platforms such as MushroomObserver.org, iNaturalist.org or MycoMap.com.

A total of 219 ITS, 122 LSU, 30 RPB2, 24 TEF1 and 11 BTUB GenBank accessions belonging to Section *Amidella* are included in this study (Online Resource 1). We excluded *Amidella* accessions MK782535 (it contains errors and was suppressed at the submitter's request), OQ754998–9 (two partial reads of the same specimen that fit badly in the LSU phylogeny), and duplicates KT779088, OP681731 and OP681759. Redundant environmental sequences in the UNITE database version 10FU (Abarenkov et al. 2024) were not included either. A Species Hypothesis code at 1.5% distance level (Kõljalg et al. 2013), was obtained for most of the ITS sequences.

Geographic references for each sequence were obtained primarily from the GenBank records, complemented by further information that could be retrieved from other sources (scientific publications, the reference website amanitaceae.org (Tulloss and Yang 2024) and online platforms).

## Outline of the phylogenetic analyses

Most ITS sequences were processed in the ITSx programme (Nilsson et al. 2010; Bengtsson-Palme et al. 2013) in the PlutoF platform (Abarenkov et al. 2010), marking the 'Concat' option to obtain the ITS1:ITS2 concatenate. Those that, for being incomplete, were left unprocessed, were later aligned to the concatenated sequences to allow the safe removal of the intercurrent 5.8S gene sequence from each one.

The decision to use the ITS1:ITS2 concatenate fell out from the surprising heterogeneity of the 5.8S sequences within *Amidella* (Table 2). This hinted at the possibility that the 5.8S gene might distort the phylogenetic analyses based on the ITS region, and although it was later realised that, as expected from the fact that most of the phylogenetic signal is from the spacer regions, the differences of including the 5.8S gene were minimal (Online Resource 2), we had two important reasons to use the ITS1:ITS2 concatenate exclusively: first, to avoid potential inconsistency in the more critical clades; second, to have a phylogeny based purely on intronic sequences, in principle not subject to evolutionary constraints that act on the exonic ribosomal sequences, to enhance the contrast with the phylogenetic analyses based on the LSU.

All alignments were made in the UGene platform version 49 (Okonechnikov et al. 2012) using the built-in MUSCLE tool version 5 (Edgar 2022), complemented by extensive visual corrections. The alignments were finally trimmed of terminal tracts with low coverage. They can be retrieved in a figshare repository, https://doi.org/10.6084/m9.figshare.26352388.

Phylogenetic reconstructions (Nei and Kumar 2000) were made mainly with MEGA11 (Tamura et al. 2021). The presence of indels (in all but the RPB2 alignment) necessitated the use of partial site deletion procedures, either pairwise deletions in the Minimal Evolution tree building method (Rzhetsky and Nei 1992), or partial deletion at minimum 67% coverage for inclusion of each site, in the Maximum Parsimony and Maximum Likelihood methods. Branch support was determined by the bootstrap method (Felsenstein 1985). Splitstree CE version 6 (Huson and Bryant 2006) was used, with default settings, for alternative network representations that allowed a rapid monitoring of new alignments, and also for Pairwise Homoplasy Index calculations (Bruen et al. 2006). Tanglegrams were constructed in Dendroscope 3.8 (Huson and Scornavacca 2012), from MEGA11 dendrograms exported in Newick format.

To evaluate which sequences presumably belong to the same species/Operational Taxonomic Units (OTUs), and to allow the application of p-distance thresholds for tentative species, dendrograms were built using the Minimal Evolution method with pairwise deletion, p-distances without corrections and uniform rates among sites (no Gamma correction). In the resulting 'tree sessions', using the 'Collapse Nodes' menu, option 'By Sequence Difference', the branches within a p-distance below a given (adjustable) threshold were condensed. The threshold was estimated for the LSU dataset using the barcode gap discovery approach (Puillandre et al. 2012). The USEARCH software, version 11, was used as an alternative approach for delimiting centroid-based sequence clusters (Edgar 2010).

## RNA structure

Using as inputs the same sequence alignments as those for the phylogenetic analyses (LSU, 5.8S, ITS1 and ITS2 regions), except for the elimination of a few sequences that failed to cover most of the alignment, and for the substitution of U for T, consensus secondary structures were obtained with RNAalifold version 2.4.3 online (http://rna.tbi.univi.e.ac.at/cgi-bin/RNAalifold.cgi, Gruber et al. (2008), last accessed July 22, 2024), with default settings including RIBOSUM scoring (Bernhart et al. 2008) excepting the use of energy parameters from the Andronescu model (Andronescu et al. 2007) for the LSU and 5.8S structures (Lorenz et al. 2011).

# Results

## Clades at subsection and stirp level

Figure 1 provides an overview of the main subdivisions within *Amidella* based on the ITS concatenates, with 5 clades with good bootstrap support that could be considered at the level of subsection: 'Volvatae', 'Lepiotidae', 'Ponderosae', 'Sagittariae' and 'Baakani'. The results also indicate further subdivisions (stirp level, Online Resource 3) in the most taxa-rich clades: Volvata, Peckiana and Fulvisquamea (Volvatae clade), Lepiotoides and Avellaneosquamosa (Lepiotidae clade), Ponderosa, Pinophila and Claristriata (Ponderosae clade). Variations in the tree building method support the same 5 main clades (Table 3, Online Resource 4a).

Except for Lepiotidae, the same clades are obtained for the LSU gene (Table 3, Online Resource 4b), and the congruence between the two phylogenies is highlighted by the tanglegram based on specimens in common between the two datasets (81 of *Amidella* and 13 of the outgroups), with a few differences in Lepiotidae involving long branches, and in the branching order within Volvatae (Figure 2). Further analysis of other markers (RPB2, TEF1, BTUB) is limited by the reduced taxa coverage, but was supportive for the main clades (Table 3 and Online Resource 4c–e), as well as in the phylogeny obtained from a concatenated LSU-RPB2-TEF1 dataset (Online Resource 4f).

Pairwise Homoplasy Index calculations showed no evidence of recombination, with $\Phi_w > 0.05$ in all alignments (Bruen et al. 2006).

## Clades at species level

A phylogenetic reconstruction based on the ITS concatenates was made without distance corrections (i.e., based on the percentage divergences calculated pairwise, and without gamma corrections for between-site heterogeneities) to allow the application of p-distance thresholds for tentative species. By adjusting the minimum distance threshold to separate species at different levels, the 1.3% level was chosen based on the fact that, for the phylogeny obtained, none of the previously recognised species were merged and very few (*Amanita rufobrunnescens*, *A. pseudovalens* and *A. hiltonii*) were split. At this level, a total of 66 branches were obtained within *Amidella* (Figure 3). For the LSU dataset the choice of a 0.81% threshold was determined by the Automatic Barcode Gap Discovery method (Puillandre et al. 2012) and applied as in the ITS concatenate dataset (Figure 4). Although in this case a few mergers occur (for example, between *Amanita lanigera* and *A. brunneomaculata*), ad-hoc attempts at lower levels were judged less satisfactory because more taxa were split. The 0.8% threshold value was also applied for the UCLUST clustering algorithm in USEARCH (Edgar 2010) to provide an alternative delimitation at species level. The phylogenies derived from ABGD and UCLUST differed only slightly from the one in Figure 4, especially in sorting out some of the merged species (Online Resource 5). The LSU dataset allowed the identification of the branches that likely correspond to *Amanita volvata*, *A. peckiana*, *A. avellaneosquamosa* and *A. clarisquamosa* (Table 1) and suggested further species, producing a complete list of 78 tentative species (Table 4). Overall, 45 clades at species level are represented by a unique specimen collection or environmental sequence, and designated "singletons".

## RNA structure analyses

The consensus RNA secondary structures for the LSU region suggest a regular solution for subclades *Volvatae*, *Lepiotidae* and *Ponderosae*, despite minor differences between them, distinct from the one obtained for the outgroup (Figure 5a). The main difference between the *Amidella* subset and the outgroup, and section *Phalloideae*, lies on pairings that have higher positional entropy (Figure 5b). Our comparison between the 5.8S *Amidella* sequences from clades I, II and III (Table 2) shows that only one stem-loop structure has a low positional entropy (Online Resource 6), while the remainder of the structure differs markedly between clades, thus producing an incongruent structure when the three clades are used simultaneously ('Amidella' in the same figure).

As expected, the degree of conservation in the ITS regions is much lower than the LSU, to the point that no consistency was found within Lepiotidae, but still suggesting a relative proximity between Volvatae and Sagittariae, on one hand, and between Ponderosae and Baakani on the other (Online Resource 7).

## Biogeography of *Amidella*

Though limited by the geographically uneven representation, the present outline of the species diversity (Table 4 and Online Resource 8) brings out noteworthy aspects:

First, the species are distributed over temperate, subtropical and tropical regions only, comprising 11 of the biogeographic regions defined by Loidi and Vynokurov (2024); the highest diversity is in Asia (38 species, almost half of the total), followed by North America (21 species), Africa (10 species), Europe (5 species) and Oceania (4 species). No sequences are known from South America, and 2 species are known (as basidiomes) to be shared between Southern Europe and North Africa (Neville and Poumarat 2004). All Baakani species are from Sub-Saharan Africa.

Second, most species have a limited geographic range. Examples with a relatively wider distribution are *Amanita fulvisquamea*, *A. volvata*, *A. ponderosa* and *A. whetstoneae*, but in some of these cases the extended range is represented by vegetative samples only: the presence of *A. ponderosa* in oak ectomycorrhizas from South England, for example, is far to the North from the known northernmost occurrence of this species as basidiomes (Neville and Poumarat 2004), and *A. fulvisquamea* basidiomes are only from Northern Thailand (Liu et al. 2023). Currently, only *A. ponderosa* and *A. pseudovalens* overlap biogeographical regions.

Finally, (Tulloss and Yang 2024) list 14 species (some with provisional names) without available sequences (Online Resource 9): seven from Africa (*Amanita floccosolivida* Beeli, *A. fulvosquamulosa* Beeli, *A. goossensiae* Beeli, *A. irreperta* nom. prov. E.-J. Gilbert, *A. olivacea* Beeli, *A. subviscosa* Beeli, *A. subviscosa* sensu Pegler & Shah-Sm.), one from Asia (*A. duplex* Corner & Bas), four from North America (*A. dolichopus* nom. prov. Tulloss, *A. fallax* nom. prov. Tulloss & G. Wright, *A. occidentalis* O. K. Mill. & D. J. Lodge, *A. pseudovolvata* nom. prov. Tulloss) and three from Oceania (*A. curta* (Cooke & Massee) E.-J. Gilbert, *A. grisea* Massee & Rodway, *A. pallidogrisea* A. E. Wood). *A. floccosolivida* and *A. pallidogrisea* may not belong to section *Amidella* (Tulloss and Yang 2024), and in that case it would be less likely to have them captured in our search; apart from that, a relation to specimens or samples for which any of the classified sequences are known seems to be possible only for *A. subviscosa* sensu Pegler & Shah-Sm. (for the Zambian 'subviscosa s.l.' species clade) and

*A. fallax* (for the ‘sp. CA03’ clade).

## Taxonomy

We first propose an update of the description of section *Amidella*, and later outline the species composition of the clades within *Amidella* (Table 4).

### Updated description for Section *Amidella*

In their taxonomic scheme for European species of *Amanita*, Neville and Poumarat (2004) adopted subsection *Ovoideinae* Singer, corresponding to the original genus *Amidella* E.-J. Gilbert, and within it they made a split between series *Ovoidea* and series *Amidella*, proposing for the first time in our knowledge a separation between the current concept of *Amidella* and species that have been replaced in section *Roanokenses* (Wolfe et al. 2012; Cui et al. 2018; Riccioni et al. 2019). Their keys to the *Amidella* series result in the following diagnosis (our translation from the original in French):

Margin of the pileus smooth and sometimes appendiculate, saccate volva under the stipe, annulus rudimentary or absent, quick discolouration of the context to reddish tones either by surface rubbing or exposure to air, later to brownish tones, amyloid spores (Neville and Poumarat 2004).

According to Neville and Poumarat (2004), the separation of the two series is for "reasons of convenience" based on the discolourations. However, for non-European species the above diagnosis and the discolouration criterium require further clarification: besides the fact that in many specimens the pileus margin is striated and not smooth, the requirement for discolouration is left to some questioning, with an "unchanging trama" commonly reported, while several species in the current concept of *Roanokenses* have discolourations considered typical of *Amidella*, for example *Amanita daucipes* (Mont.) Lloyd, *A. fulvopulverulenta* Beeli, *A. luteofolia* Yang-Yang Cui, Qing Cai & Zhu L. Yang or *A. mutabilis* Beardslee (Tulloss 1984; Cui et al. 2018; Tulloss and Yang 2024). It is only in combination with other characters, namely the saccate volva and lack of a persistent membranous ring, that the discolourations can help define *Amidella*.

Currently, at section level, *Amidella* is the taxon introduced by Konrad and Maublanc (1948), who sweepingly transferred it from the one defined by Gilbert at subgenus level. Although the taxon is not listed in the Indexfungorum (Royal Botanic Gardens Kew et al. 2024) and Mycobank (Robert et al. 2005) nomenclatural databases, it is considered valid by different authorities (Neville and Poumarat 2004; Cui et al. 2018; Tulloss and Yang 2024). However, contrary to Gilbert's nuanced original description of the protolog for genus *Amidella* (Neville and Poumarat 2004), the concept of section *Amidella* needs revision because it excludes striation of the pileus margin, which is sometimes mentioned for the section (Yang et al. 2001; Sanmee et al. 2008) and specifies a bulbous stipe base (Konrad and Maublanc 1948). Furthermore, we find that several European species show a faint grey reaction with iron sulphate on the exposed context, as suggested first by the analysis of *Amanita ponderosa* (Tulloss and Yang 2024) and in contradiction to a rule put forward for the whole genus (Neville and Poumarat 2004). We believe that this reaction should be systematically tested for the whole of *Amanita* in order to determine its phylogenetic extent. Therefore, we propose the following update:

Section *Amidella* Konrad & Maublanc mut. char. (emend. P. Oliveira & R. Arraiano-Castilho)

Type species: *Amanita volvata* (Peck) Lloyd

Agaricoid basidiomata, all parts except veil remnants uniformly coloured, initially whitish and becoming ochre to tan with aging or drying. Usually, the surface of fresh specimens quickly reddens where rubbed or bruised, in some cases with a brownish or greyish reaction. Universal veil persisting as a saccate membranous volva around the stipe base and, sometimes, as membranous patches on the pileus, with darker remnants of the inner friable layer visible on the lower part of the stipe, frequently as zebroid markings, and/or on the pileus as squamules or patches (lepiotoid appearance). Remains of the partial veil adherent to the upper part of the stipe, sometimes contrasting with the more intensely discoloured friable remains of the universal veil on the lower part, sometimes also visible in immature specimens as an evanescent creamy annulus and/or as tiny appendiculate rags on the pileus margin. Pileus margin can be striated (*Vaginatae* appearance). Lamellulae truncate to nearly truncate, lamellar edge normally concolorous. Stipe base usually not bulbous in cross section (discounting the added thickening by the volva). Context (fresh) frequently showing a quick reddening on exposure, and/or (fresh as well as dried) a faint grey reaction with iron sulphate solution. Spores amyloid, usually elongate to cylindric (typically average Q > 1.6), septa without clamps in all parts.

## Clades at subsection level

### Volvatae

The proposed name is based on the assumption that *Amanita volvata*, the type species for section *Amidella*, is represented by accession AF024485 (Table 1). At least one close LSU sequence (GenBank accession OQ311333) is from a specimen also represented in the ITS concatenate dataset, thus enabling the merging of various ITS sequences within this species. A similar merging process was based on the LSU sequences (accessions MH620300 and OQ286517) clustering with the one assumed to be *A. peckiana* (accession HQ539720, Table 1).

We note that Neville and Poumarat (2004) have used *Volvatae* at section level, following previous authors to encompass (for Europe) current sections *Amidella*, *Phalloideae*, *Validae* and part of *Roanokenses*.

Three resolved and well-supported subclades at stirp level are considered:

1. Volvata

Comprises the type species *Amanita volvata* (Peck) Lloyd, *A. clarisquamosa* (S. Imai) S. Imai (both based on the assignments in Table 1), *A. curtipes* E.-J. Gilbert, *A. pseudovalens* (Neville & Poumarat) Arraiano-Castilho et al., and 3 undescribed or provisional species (Table 4). According to our analyses, a variety of provisional names from the Eastern United States (sp. T52 Tulloss & S. D. Russell, IN57, IN36) are all *A. volvata*.

2. Peckiana

Comprises *Amanita peckiana* Kauffman in Peck, *A. brunneomaculata* Yang-Yang Cui, Qing Cai & Zhu L. Yang, and *A. lanigera* Yang-Yang Cui, Qing Cai & Zhu L. Yang (Table 4). According to our analyses, *A. floridella* Tulloss & S. D. Russell nom. prov. is *A. peckiana*.

3. Fulvisquamea

Comprises *Amanita fulvisquamea* Yuan S. Liu & S. Lumyong and 1 undescribed species. The latter is represented by a single LSU sequence that clusters in this subclade with weak support (Table 3).

This subclade clusters with Peckiana only for the ITS concatenate dataset (figures 2–4, Online Resource 4a–c), thus we consider it separately in our analyses.

## Ponderosae

The proposed name is based on the oldest accepted species in this clade, *Amanita ponderosa*. Three well-resolved and well-supported subclades at stirp level, and an unclassified LSU sequence, are considered:

1. Ponderosa

Comprises *Amanita ponderosa* Malençon & R. Heim and *A. whetstoneae* Tulloss, Goldman and Kudzma nom. prov..

2. Pinophila

Comprises *Amanita pinophila* Yang-Yang Cui, Qing Cai & Zhu L. Yang and an unidentified specimen from China labelled *A.* aff. *albidostipes*, an epithet that applies to a species in Section *Vaginatae* (Cui et al. 2018).

3. Claristriata

Comprises *Amanita claristriata* Yuan S. Liu & S. Lumyong, *A. neocaesariensis* Tulloss, I. Safonov & K. W. Hughes nom. prov. (including specimens labelled IN26), and around 6 other undescribed species. The latter uncertainty is due to the limited species-level correspondence between some ITS clades (sp. OMS-010/aff. volvata6, aff. volvata7, aff. volvata8, sp. S19/aff. volvata9) and their presumed LSU counterparts (see Discussion).

4. Unclassified:

*Amanita* sp. Arora-01-560 cryptonom. temp. is represented by an LSU sequence that appears, in the unrooted radiation tree, as a very long basal branch between the three subclades outlined above (Online Resource 4b) that lowers the bootstrap support for *Ponderosae* considerably (Table 3). A fourth subclade at stirp level might be considered in the future, as more sequences become known.

## Lepiotidae

The proposed name is based on the oldest accepted epithet present, *Amanita lepiotoides*. This clade is by far the most species-rich (about half of Section *Amidella*), and the scheme considered here at stirp level (Table 4) is a simplification of what could amount to some 8 subclades. For the ITS concatenate the Lepiotoides name is given to a monophyletic group with low support, while for the LSU dataset it is paraphyletic (Table 3, Figure 2, Online Resource 4a-b); on the other hand, good LSU support to a monophyletic Avellaneosquamosa at stirp level contrasts with its low support in the ITS dataset, because of several intermediate branches (Table 3, Online Resources 3/4a-b).

Moreover, there is a potential difficulty with the names to be used. The available sequences named *Amanita lepiotoides* are of the forma *subcylindrospora* (Neville and Poumarat 2004; Arraiano-Castilho et al. 2022) and one cannot be certain whether the autonym would be phylogenetically close or not. Furthermore, as pointed out in the footnote to Table 1, the original *A. avellaneosquamosa* could be different from our assumption, noted here as 'cf. avellaneosquamosa 1' (Table 4, Online Resource 1 and Figures 3–4). Bearing this in mind, if any of these two taxa turn out to belong outside the clades under consideration, the respective naming should be revised.

Based on the ITS concatenate phylogeny, two subclades are considered at stirp level:

1. Lepiotoides

Comprises *Amanita lepiotoides* f. *subcylindrospora* Neville & Poumarat, *A. rufobrunnescens* W. Q. Deng, T. H. Li & Hosen, *A. parvirufobrunnescens* A. Kumar, Y. P. Sharma & Mehmood, *A. pseudorufobrunnescens* K.C. Semwal, K. Das, R.P. Bhatt, Mehmood & V.K. Bhatt, *A. insolens* I. Safonov & L. V. Kudzma nom. prov., *A. texidella* Tulloss, Pastorino & K. W. Hughes nom. prov., and 21 other species, including possibly *A. subviscosa* Beeli (Table 4). The LSU sequence named *A.* aff. *goossensiae* KM 12 has a position in this subclade that appears not to be the one expected for the clade *Baakani* (see below), where other specimens also bear the epithet also as approximation. The *A. subviscosa* s.l. clade groups three geographically segregate SH codes that collapse at 1.4%, and it is uncertain whether any of these correspond to *A. subviscosa* (Table 4, see also Discussion). One of the LSU sequences (accession MH620228) is named *A. mutabilis*, a species with a membranous ring belonging in Section *Roanokenses* (Tulloss & Yang 2024), thus the clade is named *A.* aff. *mutabilis* instead.

2. Avellaneosquamosa

Comprises 14 clades, including *Amanita parvicurta* Yang-Yang Cui, Qing Cai & Zhu L. Yang, the specimen presumed to represent the original *A. avellaneosquamosa* (S. Imai) S. Imai in Ito, included here in clade A. cf. avellaneosquamosa1 (see note on Table 1), and 12 undescribed species. The epithet *rosea*, given with a cf. to one LSU sequence (accession OQ628446), is of a species belonging in Section *Roanokenses*, and for this reason *A.* aff. *rosea* is used to name the corresponding clade.

## Sagittariae

This clade is composed of 8 singletons, and named after *Amanita sagittaria* Tulloss & K. W. Hughes nom. prov.. Two sequences are named *A. clarisquamosa*, but the specimen assumed to represent this species (Table 1) has an LSU sequence in the *Volvatae* clade, and given the position of the Sagittariae specimens represented by both ITS and LSU sequences, it appears to be unrelated (Figure 4 and Online Resource 4b); therefore, the *clarisquamosa* epithet is questionable in clade Sagittariae.

## Baakani

This clade is named with reference to the Ba'Aka nation in Cameroon, who live in the provenance of six undescribed specimens named for the apparent affinity to *Amanita gossensiae* Beeli, a species classified in Section *Amidella* (Tulloss and Yang 2024). The position of the latter in this clade still needs to be demonstrated, and a separate collection named *A.* aff. *goossensiae* KM 12, also from that provenance, with an LSU sequence close to two other African collections within *Lepiotidae* (see above), might be an alternative candidate for *A. goossensiae*. In addition to the Cameroonian specimens, grouped in two clades at species level, there are three additional sequences from Zimbabwe collections with separate SH codes at 1.5%, which are here considered as representing two species.

## Hesleri

This clade has a position that suggests being closer to Section *Amidella* than the outgroup (Figure 1,

Online Resource 3), even clustering with the *Baakani* clade in the Maximum Parsimony ITS tree (Online Resource 4a). However, *Amanita hesleri* Bas and *A. zangii* Zhu L. Yang, T.H. Li & X.L. Wu are placed within Section *Phalloideae* (Cai et al. 2014; Cui et al. 2018), and since their morphologies do not conform to the established concept of Section *Amidella*, namely for the lack of saccate volva and lack of discolourations of the basidiome, as well as for the microscopy of the volval remnants on the pileus (Yang et al. 2001; Tulloss and Yang 2024), their placement (and of the Baakani clade) had to be examined, both for the ITS1:ITS2 concatenate and the LSU (Online Resource 10). The Hesleri clade clusters within *Phalloideae*, while Baakani clusters with the *Amidella* sequences (Cui et al. 2018). The short branches in Hesleri may be the reason why these accessions were retrieved in our BLAST-based searches. We judged worth keeping this clade in our analyses to represent the branching point of Section *Phalloideae*.

## Discussion

The present work presents for the first time, as far as the authors are aware, a nearly exhaustive sampling of the DNA sequence diversity available for section *Amidella*. Our analyses demonstrate an unsuspected structure, defining five clades at subsection level, three of which further subdivided at stirp level (Fig. 1, Online Resource 3). This structure can be very useful in collecting sequences for phylogenetic analyses more effectively, both by overriding the need for very large datasets in studies of closely related groups (Dissanayake et al. 2024) and helping form a balanced representation of the groups to be studied. Although well-supported in general (the main exception is a partial disruption of Lepiotidae in the LSU phylogenies, Figure 2, Online Resource 4b, Table 3), their relative topologies depend on the phylogeny: thus, Volvatae and Lepiotidae are sister groups for the protein-coding genes, but Lepiotidae and Ponderosae for the ITS concatenate. Future multigene sequencing efforts aimed at increasing the coverage of section *Amidella* with protein-coding genes may help resolve the branching order at subsection level. With the limited information available, the hinted placement of Sagittariae as sister clade to Volvatae and of Baakani as sister clade to Ponderosae is exploratory.

The complexity within *Amidella* is also expressed by a total of about 78 clades at species level (Figs. 3–4), of which only up to 17 have accepted names (Table 4). The delimitation of clades at species level was done with a branch clustering option in MEGA, using consensus cutoffs for the pairwise uncorrected p-distances that, in this group, were set at 1.3% for the ITS concatenate and 0.81% for the LSU. This approach is tree-based and is not limited by the high prevalence of singletons, which is an obstacle for statistical approaches that rely on intraspecies diversity such as sGMYC or mPTP (Fujisawa and Barraclough 2013; Leavitt et al. 2015; Sánchez-Ramírez et al. 2017; Maharachchikumbura et al. 2021). However, the cutoff used for a given phylogeny is likely heterogeneous in different branches, as suggested by the slight differences between different approaches to the LSU dataset (Figures 4 and S3). It has been suggested that only the ITS2 region has an effective barcoding gap for *Amanita* (Badotti et al. 2017). At any rate, the evidence of a high number of novel species, mainly in clades Baakani, Sagittariae, Lepiotidae (subsection level) and Claristriata (stirp level), is compelling.

It is worth mentioning that, because of nrDNA cistron heterogeneity, a single sequence might not be representative of the collection itself (as so well exemplified in Figure 3 and Online Resource 4a with the *Amanita hiltonii* ITS sequences in the outgroup (Davison and Giustiniano 2020)). Genomic analyses have demonstrated that the same can happen in the LSU (Paloi et al. 2022; Cedeño-Sanchez et al. 2024). Nevertheless, a recent survey has obtained a >99% pairwise identity between ITS cistrons in *Amanita* (Bradshaw et al. 2023), therefore we feel that nrDNA cistron heterogeneity would affect, if anything, the separations at interspecies level rather than higher-level relationships. Thus, the total number of estimated species might be more subject to revision than the 5 main subclades at subsection level.

Unfortunately, the sequences are the only support for these subclades. Even with the very detailed descriptions available (Deng et al. 2016; Cui et al. 2018; Semwal et al. 2020; Liu et al. 2023; Tulloss and Yang 2024), our attempts to derive morphology-based simplesiomorphies were unsuccessful. On

the other hand, the available alignments suggest efficient means to retrieve enough sequence information from type specimens to resolve the hitherto unclear identification of taxa such as *Amanita avellaneosquamosa* (Table 1; Online Resource 11).

Practically all species have, strikingly, a relatively narrow geographical range (Table 4, Online Resource 8), as reported already for other taxa (Looney et al. 2016, 2020; Bazzicalupo et al. 2019; Noffsinger et al. 2024). This could help avoid using the same name for geographically distinct collection origins — as the clade naming used here at species level demonstrates (Table 4), the *volvata* and *avellaneosquamosa* epithets have been used very confusingly. Three contrasting examples serve to highlight the application of this geographic premise, and difficulties that may arise:

i. The clade *A. subviscosa* s.l. spans an unusually vast distribution in Africa, from Benin to Zambia and Zimbabwe (Table 4). We named it so for two reasons: first, the SH codes at 1.5% are unique for each country, thus suggesting that three species of narrower range are to be considered; second, *A. subviscosa* has been described from a fourth country (Online Resource 9) and is regarded as different from an unsequenced collection from Zambia (Tulloss and Yang 2024), therefore one cannot be sure which of the sequenced specimens, if any, represents *A. subviscosa*.
ii. There are two North American clades in Claristriata (Ponderosae) with contiguous geographical distributions: *Amanita* sp. S19/aff. volvata9 ranging from Massachusetts to Tennessee, and A. sp. T51 in Mississippi and Eastern Texas. They collapse into a single lineage at 1.45% divergence, raising the possibility that they could be a single species, but their disjunct distributions, in view of their close relationship, could be interpreted as vicariant. Still with the *Amanita* sp. S19/aff. volvata9 group, there is an LSU sequence from a South Korean collection that is very close phylogenetically. An ITS sequence for this collection would help understand its relationship with the North American group, but none is available. Tentatively, we consider the South Korean collection autochthonous and assign a separate epithet (*Amanita* sp. volvata8), but it could turn out to be the same species as the North American collections, and one possible explanation could be a human-driven migration, as a carryover from host transplantation originating in the United States.
iii. The provenances of Eastern Asia (specifically, Mainland China, Taiwan, Japan and South Korea) have only a minority of shared species (Online Resource 12), most of them between Japan and South Korea, while Mainland China shares only 2 out of 16 with Japan (one being *Amanita* aff. avellaneosquamosa3/OMS-012, but the Chinese occurrence is in a botanical garden in Jianxi; the other is *A. fulvisquamea*, a rather wide-ranging species when soil and root tip samples are considered, however apparently fruiting only in Thailand). One should expect these separations to blur as more findings are reported, but currently, for any species in this group, it is worth considering with caution a shared geographical distribution between Mainland China and Japan, as we note on Table 1.

A considerable part of the species deduced from clades in the present work (twelve, to be precise) are represented by LSU sequences without an ITS counterpart. This raises two questions: i) is any of these species the same as one of those defined by ITS sequences without LSU counterpart, such that both should be merged, and ii) is the species threshold used for LSU (0.81% p-distance, Figure 4)

anywhere less applicable?

To answer the first question, it narrows down to just three possible pairs classified in the same subclade and not too far geographically: in Claristriata, one could consider *Amanita* aff. avellaneosquamosae6 (ITS; China, Yunnan) and *A.* aff. volvata8 (LSU; South Korea), but the latter is phylogenetically very close to *A.* sp. S19/aff. volvata9 (Eastern United States) and the former is not; in Avellaneosquamosa, *Amanita* aff. rosea (LSU) is Australian like *Amanita* sp. 19 GMB-2014 (ITS) and both are phylogenetically close to *Amanita parvicurta*, but the first is from New South Wales and the second from Northern Territory; in Lepiotoides, the origins of *Amanita* sp. clone d28 3Ama (ITS; China, Eastern Himalaya) and *A.* aff. volvata1 (LSU; China, Yunnan) are similar, but their relationships with other clades present in both phylogenies (*Amanita subviscosa* s.l., *A.* sp. Thai04, *A.* sp. JPN01/sp. OMS-015) are incongruent. Furthermore, one must consider the *Amanita* sp. S17/sp. IN41 clade. A single publicly available LSU accession (HQ539665) is reported to correspond to the reference specimen RET 444-9 (Tulloss and Yang 2024), but several others in the ITS concatenate dataset are identified as *Amanita* sp. S17, in a cluster that includes specimens designated *A.* sp. IN41, *A.* "volvata" or *A.* "peckiana". This extension of *Amanita* sp. S17 seems reasonable since all specimens are from Eastern United States, with similar ecology and phenology and, like accession HQ539665, they cluster with the same group of clades at species level (*Amanita* aff. volvata5, *A.* aff. avellaneosquamosa4/aff. volvata3, *A.* cf. avellaneosquamosa1).

The second question highlights a contrast between the criterium used here for the ITS (1.3%, Figure 3) and the one used for the LSU phylogeny (0.81%, Figure 4). While the former was chosen by trial and error, guided by branch collapsing within well-defined species, for the latter there was no consensus value that could mimic the results with the species also present in the ITS concatenate dataset. Thus, we used the ABGD method (automatic barcoding gap discovery) to obtain the 0.81% estimate for the p-distance to be used for LSU and ran UCLUST using a similarity level of 99.2% to have a phylogeny-independent approach to delimiting sequence clusters. From both approaches it was possible to obtain LSU phylogenies (Online Resource 5) not unlike the one in Figure 4, separating adequately some of the merged clades in the latter, for example the one containing *Amanita lanigera* and *A. brunneomaculata*. We take these results as independent support for the proposed species list (Table 4), but it is admissible that part of those twelve LSU-only clades might be considered for merging with others, especially as the respective ITS sequences become known.

We wanted to explore the possible utility of RNA secondary structure reconstructions as a new level of phylogenetic support, as has been done in a variety of contexts (Zhuang and Liu 2012; Kapoor et al. 2018; Sundaresan et al. 2019). Our use of RNAalifold (Bernhart et al. 2008) aimed at verifying, within the framework of the collected sequences, whether the taxonomic results from the one-dimensional (sequence-only) analyses could be reinforced by working with the secondary structures. The authors are fully aware that the secondary structures obtained may not be those that function *in vivo*, since only a part of the 28S nrRNA was available, and also because the 5.8S nrRNA is known to form paired structures with the 5' end of the 28S (Ursi et al. 1983), which are excluded from consideration when analysed separately as we did. For the purpose of this study, the RNAalifold outputs are consensus structures that provide a framework for judging the variation within each

taxonomic group and the tendency for divergence between groups, colour coded as a function of sequence conservation within the alignment and of the positional entropies on each part of these consensus structures (Figure 5). This is a rather simple procedure that allowed an alternative view on the taxonomical subdivision proposed for section *Amidella*. It also confirmed the disparities in the 5.8S gene (Table 2 and Online Resource 6). For studying the variation in the ITS regions (Online Resource 7) this approach seemed less useful.

The present study reveals the dimension of some knowledge gaps regarding section *Amidella*: a large majority of undescribed species, a sizeable number of described species without molecular information, the need for molecular studies on types to clarify the identity of species such as *Amanita avellaneosquamosa* and *A. clarisquamosa*, an overwhelming need for novel discriminant characters, the utility of which being from now on framed by the infrasectional taxonomic structure.

One final point. In naming the clades, we had no choice but to use three epithets (*fulvisquamea*, *claristriata*, *pinophila*) that have been accepted only recently (Cui et al. 2018; Liu et al. 2023), *sagittaria* that is still provisional (Tulloss and Yang 2024), and devise a novel name for the Baakani clade. It is clear that section *Amidella* is still a largely untrodden field of research.

## Author Contributions

Both authors made substantial contributions to the conception of the work, and to the acquisition, analysis, and interpretation of data; both authors drafted the work and revised it critically for important intellectual content; both authors approved the version to be published; and both authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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## Disclosure

The authors have no relevant financial or non-financial interests to disclose.

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Table 1. Outline of the nucleotide accessions representing names that are frequently misapplied.

| Current name (basionym) | GenBank accessions | Authority |
|---|---|---|
| *Amanita volvata* (*Agaricus volvatus* Peck 1872) | AF024485 | Tulloss, R. E. http://www.amanitaceae.org/?Amanita%20volvata |
| *Amanita peckiana* (*Amanita peckiana* Kauffman 1913) | HQ539720 | Tulloss, R. E. http://www.amanitaceae.org/?Amanita%20peckiana |
| *Amanita avellaneosquamosa* (*Amanitopsis avellaneosquamosa* S. Imai 1933)[A] | MH508259<br>AF024441<br>MH486380<br>MH508682 | Tulloss, R. E. http://www.amanitaceae.org/?Amanita%20avellaneosquamosa and Cui et al. (2018) |
| *Amanita clarisquamosa* (*Amanitopsis clarisquamosa* S. Imai 1933)[A] | AF024448 | Yang, Z. L. & Tulloss, R. E. http://www.amanitaceae.org/?Amanita%20clarisquamosa and Cui et al. (2018) |

[a] The choice for *Amanita avellaneosquamosa* is particularly difficult. Of the 6 specimens included in (Cui et al. 2018) under this name, 5 separate clades were found in the present study. Furthermore, the sequence from specimen HKAS 38300, highlighted in http://www.amanitaceae.org/?Amanita%20avellaneosquamosa, is quite distant from all other *A. avellaneosquamosa*, as already noted in (Cui et al. 2018). The accession AF024441 also considered by (Tulloss and Yang 2024) is more consensual, but its origin (Yunnan, in China) is rather different from that of the type (Japan; incidentally, the same happens with the LSU sequence chosen here to represent *A. clarisquamosa*). Thus, the clade hereby chosen as reference for *A. avellaneosquamosa* is named 'cf. avellaneosquamosa1', and more generally we caution that the reference sequences listed in this Table 1 are subject to revision.

Table 2. Major clades derived from the 5.8S phylogeny with known *Amidella* species epithets. Only the epithets of accepted species are italicised, and those in double quotes are probably misapplied.

| Clade | Number of accessions | known species epithets (*Amidella*) | nearest species epithets (examples) |
|---|---|---|---|
| I | 53 | *lanigera*, *brunneomaculata*, floridella, *curtipes*, *pseudovalens*, sagittaria, "clarisquamosa", *rufobrunnescens* | *goossensfontanae*, *proxima*, *cinereoconia*, *fulvopulverulenta*, *yenii*, *hiltonii* |
| II | 81 | *ponderosa*, *pinophila*, whetstoneae, neocaesariensis, "volvata", "avellaneosquamosa", "goossensiae" | *quenda*, *daucipes*, *walpolei*, *ovoidea* |
| III | 30 | "avellaneosquamosa", *parvicurta*, "volvata", "peckiana" | *neo-ovoidea*, *preissii* |

Table 3. Comparison of bootstrap support values for the clades[a]

| Marker | ITS1:ITS2 | | | LSU | | RPB2 | | TEF1 | | BTUB | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Method | ML | ME | MP | ML | MP | ML | MP | ML | MP | ML | MP |
| Volvatae | 93 | 82 | 74 | 82 | 78 | 72 | 81 | 57 | 46 | 94 | 84 |
| Volvata | 99 | 96 | 99 | 92 | 91 | s | s | — | — | — | — |
| Peckiana | 63 | 54 | 74 | 91 | 76 | 98 | 99 | 100 | 100 | 96 | 96 |
| Fulvisquamea | m | m | m | 11 | 36 | m | m | m | m | s | s |
| Ponderosae | 99 | 68 | 99 | 38 | 60 | 100 | 89 | 100 | 91 | 82 | 52 |
| Ponderosa | 99 | 97 | 99 | 99 | 98 | m | m | — | — | — | — |
| Pinophila | 99 | 99 | 99 | m | m | m | m | m | m | m | m |
| Claristriata | 99 | 96 | 99 | 82 | 80 | m | m | m | m | m | m |
| Lepiotidae | 73 | 84 | 96 | p! | p! | 29 | p! | — | — | — | — |
| Lepiotoides | 41 | 35 | 61 | p! | p! | m | m | — | — | — | — |
| Avellaneosquamosa | 19/65[b] | 42/48[b] | p!/73[b] | 70 | 78 | 58 | 57 | 89 | 79 | 98 | 92 |
| Sagittariae | 48 | 71 | p! | 46 | 59 | — | — | — | — | — | — |
| Baakani | 99 | 99 | 99 | — | — | — | — | — | — | — | — |
| Hesleri | 99 | 99 | 99 | 100 | 100 | m | m | m | m | — | — |
| [outgroup] | 90 | 92 | 99 | 100 | 100 | 88 | 82 | 98 | 94 | 98 | 98 |

[a] m: one species, monophyletic; p!: paraphyletic; s: single sequence; — no data.

[b] general support/support for the 'core' without parvicurta, sp. canadensis01/sp. IN28, and sp. 19 GMB-2014.

Table 4. List of the clades at species level, ordered by subsection and stirp level clades.

| Subsection | Strip | Species | SH (1.5%) | Geography | Ecology |
|---|---|---|---|---|---|
| Volvatae | Volvata | clarisquamosa | | Asia, China, Yunnan | |
| | | aff. curtipes1 | SH0932681.10FU | Asia, South Korea | |
| | | pseudovalens | SH0932676.10FU | Europe, Portugal/France; Africa, Morocco | |
| | | curtipes | SH0932674.10FU | Europe, Portugal/Spain | Mediterranean, sand |
| | | sp. QU06 | SH0932677.10FU | NA, Canada, Québec | |
| | | sp. whartonensis | SH0932675.10FU | NA, USA, New Jersey | |
| | | volvata | SH0932680.10FU | NA, USA, Texas/Louisiana/ Mississipi/Indiana/New Jersey/New York | conifers/hardwoods, sand |
| | Peckiana | brunneomaculata | SH0932678.10FU | Asia, China, Yunnan | Pinus |
| | | lanigera | SH0932679.10FU | Asia, China, Yunnan | Pinus/Fagaceae |
| | | peckiana | SH0932672.10FU | NA, USA, Florida/Georgia/ West Virginia/New York | Pinus, sand |
| | Fulvisquamea | sp. LAM 0192 | | Asia, Malaysia, Sarawak | Dipterocarps |
| | | fulvisquamea | SH0932673.10FU | Asia, Thailand/China/ Taiwan/Japan | Dipterocarps/Pinus |
| Ponderosae | | sp. Arora-01-560 | | Africa, Zambia, Copper Belt Province | |
| | Ponderosa | ponderosa | SH0916963.10FU | Europe, Portugal/Spain/France/Italy/UK | Quercus/Cistus |
| | | sp. whetstoneae | SH0916957.10FU | NA, USA, Mississipi/Missouri/ Tennessee/North Carolina/ West Virginia/District of Columbia/Pennsylvania/New Jersey/New York/Indiana/Ohio | |
| | Pinophila | aff. albidostipes | SH0916959.10FU | Asia, China, Jiangxi | |
| | | pinophila | SH0916953.10FU | Asia, China, Yunnan | Pinus |

| Subsection | Strip | Species | SH (1.5%) | Geography | Ecology |
|---|---|---|---|---|---|
| Ponderosae | Claristriata | aff. avellaneosquamosa6 | SH0916962.10FU | Asia, China, Yunnan | |
| | | sp. OMS-010/aff. volvata6 | SH0916961.10FU | Asia, Japan/South Korea | |
| | | aff. volvata7 | SH0916952.10FU | Asia, South Korea, Gangwon Province | |
| | | aff. volvata8 | | Asia, South Korea | |
| | | claristriata | SH0916955.10FU | Asia, Thailand, Chiang Mai Province | Dipterocarps |
| | | sp. neocaesariensis/sp. IN26 | SH0916954.10FU | NA, USA, New Jersey/New York/Massachusetts/Indiana/ Wisconsin | Pinus |
| | | sp. S19/aff. volvata9 | SH0916956.10FU | NA, USA, Tennessee/North Carolina/Virginia/New Jersey/Massachusetts | Quercus/Carya |
| | | sp. T51 | SH0916960.10FU | NA, USA, Texas/Mississippi | mixed woods |
| Lepiotidae | Lepiotoides | subviscosa s.l. | SH0846553.10FU | Africa, Benin | |
| | | subviscosa s.l. | SH0846554.10FU | Africa, Zimbabwe | Fabaceae |
| | | subviscosa s.l. | SH0846552.10FU | Africa, Zambia, Mufumbwe district | |
| | | aff. goossensiae KM 12 | | Africa, Cameroon, Dja Biosphere Reserve | Gilbertiodendron/Uapaca |
| | | sp. TANZ01 | | Africa, Tanzania, Iringa | Fabaceae (Miombo), red soil |
| | | clone d28 3Ama | SH1013321.10FU | Asia, China, Eastern Himalaya | Polygonum |
| | | rufobrunnescens | SH0858857.10FU | Asia, China, Guangdong | Castanopsis |
| | | aff. volvata1 | | Asia, China, Yunnan | |
| | | parvirufobrunnescens | | Asia, India, Jammu & Kashmir | Pinaceae |
| | | pseudorufobrunnescens | | Asia, India, Uttarakhand | Quercus |
| | | aff. volvata2 | | Asia, Japan, Yakushima Island | Fagaceae |
| | | sp. JPN01/sp. OMS-015 | SH0891149.10FU | Asia, Japan/South Korea | Pinus |
| | | sp. LAM 0191 | | Asia, Malaysia, Sarawak | Dipterocarps |
| | | sp. TUF116503 | SH1013317.10FU | Asia, Thailand, Chiang Mai | |

| Subsection | Strip | Species | SH (1.5%) | Geography | Ecology |
|---|---|---|---|---|---|
| Lepiotidae | Lepiotoides | sp. Thai04 | SH1013318.10FU | Asia, Thailand, Isan | Dipterocarps |
| | | clone Ama3 | SH1013320.10FU | Asia, Thailand, Kamphaeng Phet | Dipterocarpus |
| | | sp. soil subtropical China 32 | | Asia, China | |
| | | sp. soil subtropical China 33 | | Asia, China | |
| | | lepiotoides f. subcylindrospora | SH0891145.10FU | Europe, Spain/France | Fagaceae |
| | | sp. QU05 | SH0891151.10FU | NA, Canada, Québec | conifers/hardwoods, sand |
| | | sp. jaliscensis | SH1013319.10FU | NA, Mexico, Jalisco | Quercus/Pinus |
| | | sp. CA03 | SH0944450.10FU | NA, USA, California | Quercus |
| | | aff. mutabilis | SH0944451.10FU | NA, USA, Florida | Quercus? |
| | | sp. IN33 | SH0858858.10FU | NA, USA, Indiana | |
| | | sp. insolens | SH0858859.10FU | NA, USA, Indiana/New Jersey | |
| | | sp. IN25 | SH0891156.10FU | NA, USA, Indiana | Quercus, sand |
| | | sp. texidella | | NA, USA, Texas | mixed woods |
| | Avellaneosquamosa | aff. avellaneosquamosa2 | SH0891153.10FU | Asia, China, Anhui | mixed woods |
| | | parvicurta | SH0891146.10FU | Asia, China, Fujian/Yunnan | Fagaceae |
| | | cf. avellaneosquamosa1 | SH0891147.10FU | Asia, China, Yunnan | Pinus |
| | | aff. avellaneosquamosa3/OMS-012 | SH0891155.10FU | Asia, China/Japan | conifers? |
| | | aff. avellaneosquamosa4/aff. volvata3 | SH0891142.10FU | Asia, Japan/South Korea | Fagaceae |
| | | aff. volvata4 | SH0891148.10FU | Asia, South Korea | |
| | | aff. volvata5 | SH0891144.10FU | Asia, South Korea, Gyeonggi | Hardwoods |
| | | aff. avellaneosquamosa5 | SH0891141.10FU | Asia, Taiwan, Nantou County | Quercus/Pinus |
| | | sp. canadensis01/sp. IN28 | SH0891154.10FU | NA, USA, Connecticut/Indiana | Quercus/Carya/Pinus |
| | | sp. IN31 | SH0891143.10FU | NA, USA, North Carolina/New York/Indiana | |
| | | sp. S17/sp. IN41 | SH0891150.10FU | NA, USA, Virginia/Pennsylvania/New Jersey/New York/New Hampshire/Massachusetts/ Indiana/Wisconsin | Hardwoods |

| Subsection | Strip | Species | SH (1.5%) | Geography | Ecology |
|---|---|---|---|---|---|
| Lepiotidae | Avellaneosquamosa | aff. rosea | | Oceania, Australia, New South Wales | Eucalyptus |
| | | sp. 12 GMB-2014 | SH0891152.10FU | Oceania, Australia, Northern Territory | |
| | | sp. 19 GMB-2014 | SH1029735.10FU | Oceania, Australia, Northern Territory | Eucalyptus |
| Sagittariae | | aff. clarisquamosa2 | SH0884338.10FU | Asia, China, Hunan | |
| | | aff. clarisquamosa1 | SH0884339.10FU | Asia, China, Yunnan | |
| | | sp. OMS-013 | SH0884342.10FU | Asia, Japan, Tokyo | |
| | | sp. OMS-017 | SH0884337.10FU | Asia, Japan, Tokyo | |
| | | sp. montelongense | | Europe, Portugal, Minho | Quercus |
| | | aff. vaginata | SH0884340.10FU | NA, USA, North Carolina | |
| | | sp. sagittaria | SH1158201.09FU | NA, USA, Florida | Pinus/Quercus |
| | | sp. 17 GMB-2014 | SH1031636.10FU | Oceania, Australia, Northern Territory | |
| Baakani | | aff. goossensiae | SH0952146.10FU | Africa, Cameroon, Dja forest | |
| | | cf. goossensiae | SH0883755.10FU | Africa, Cameroon, Dja forest | |
| | | sp. Sharp2816 | SH0883752.10FU | Africa, Zimbabwe | Caesalpiniaceae |
| | | sp. naletali s.l. | SH0883753/4.10FU | Africa, Zimbabwe, Shangangwe | |

Fig. 1. Phylogenetic tree for the ITS concatenate dataset, obtained with the Maximum Likelihood Method and General Time Reversible nucleotide substitution model (partial deletion at 67% coverage cutoff). Major branches have been compressed to provide an overview of the clades at subsection level, with bootstrap support values above 50% shown next to the branches. The scale bar is for substitutions per site.

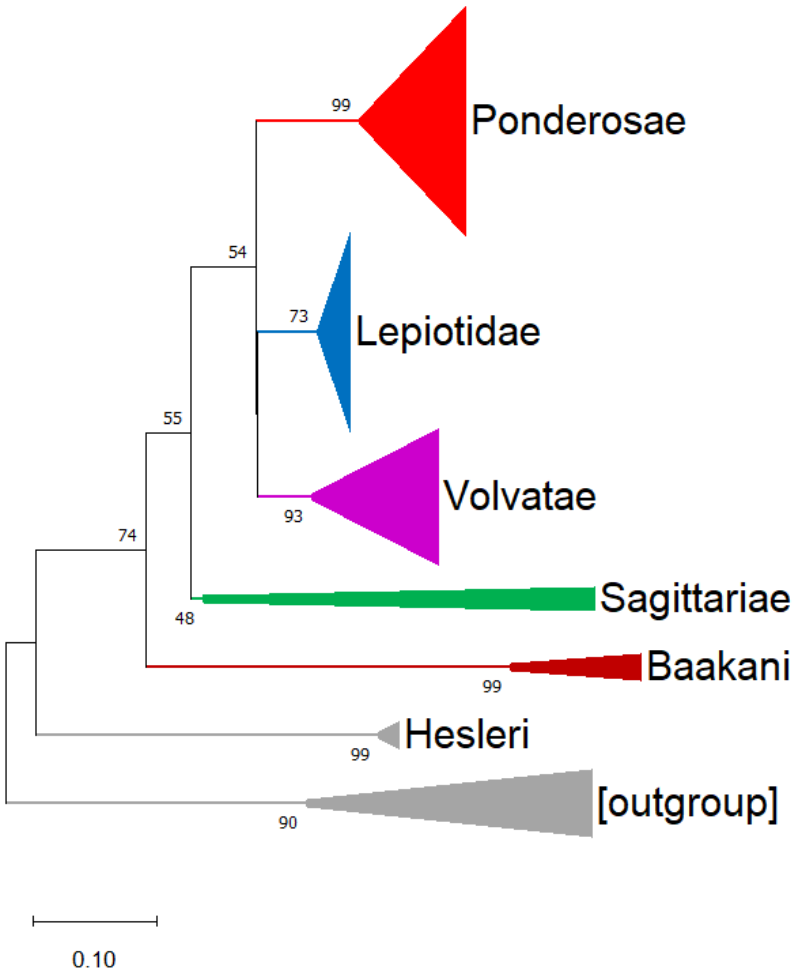
Fig. 2. Tanglegram comparing the phylogenies for the ITS concatenate and LSU datasets, uniting the branches for the same specimens at the middle. Clades at subsection level are indicated by larger circles, and smaller circles indicate clades at stirp level. Circle colours are as in Figure 1. Each tree was obtained with the Maximum Likelihood Method and General Time Reversible nucleotide substitution model (partial deletion at 67% coverage cutoff), with bootstrap support values shown next to the branches. Sequence identifiers were adapted so that a perfect match existed between the two datasets. The scale bars are for substitutions per site.

Fig. 3. Phylogenetic tree for the ITS concatenate dataset, obtained with the Minimum Evolution Method and based on p-distances without gamma correction (pairwise deletion), with bootstrap support values above 50% shown next to the branches. All branches collapsing at 1.3% distance are compressed and named with an epithet (Table 4). Remainder branches identified by the Genbank accession number and original epithets/codes. The scale bar is for substitutions per site.

Fig. 4. Phylogenetic tree for the LSU dataset, obtained with the Minimum Evolution Method and based on p-distances without gamma correction (pairwise deletion), with bootstrap support values above 50% shown next to the branches. All branches collapsing at 0.81% distance are compressed and named with an epithet (Table 4). Remainder branches identified by the Genbank accession number and original epithets/codes. The scale bar is for substitutions per site.

Fig. 5. RNAalifold minimal free energy results for different LSU RNA alignments. The free energy of each thermodynamic ensemble is given under the label next to the graphical representation of the consensus structure. a) Conservation annotation for the main *Amidella* clades at subsection level and for the outgroup. The colour coding is given on the left: dark red for base pairs of a single type, without incompatible nucleotides over the given sequences; other colours denote increased base pair diversity, and colour fading denotes the presence of one or two incompatibilities. Blue arrows indicate the 5' end of each alignment. b) Positional entropy for the section-level alignments (including, in *Amidella*, the two sequences belonging in Sagittariae). 'Rainbow' colour coding applies also to unpaired positions, dark red for minimum and violet for maximum positional entropy.

Fig. 1


Ponderosae
Lepiotidae
Volvatae
Sagittariae
Baakani
Hesleri
[outgroup]
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54
73
55
93
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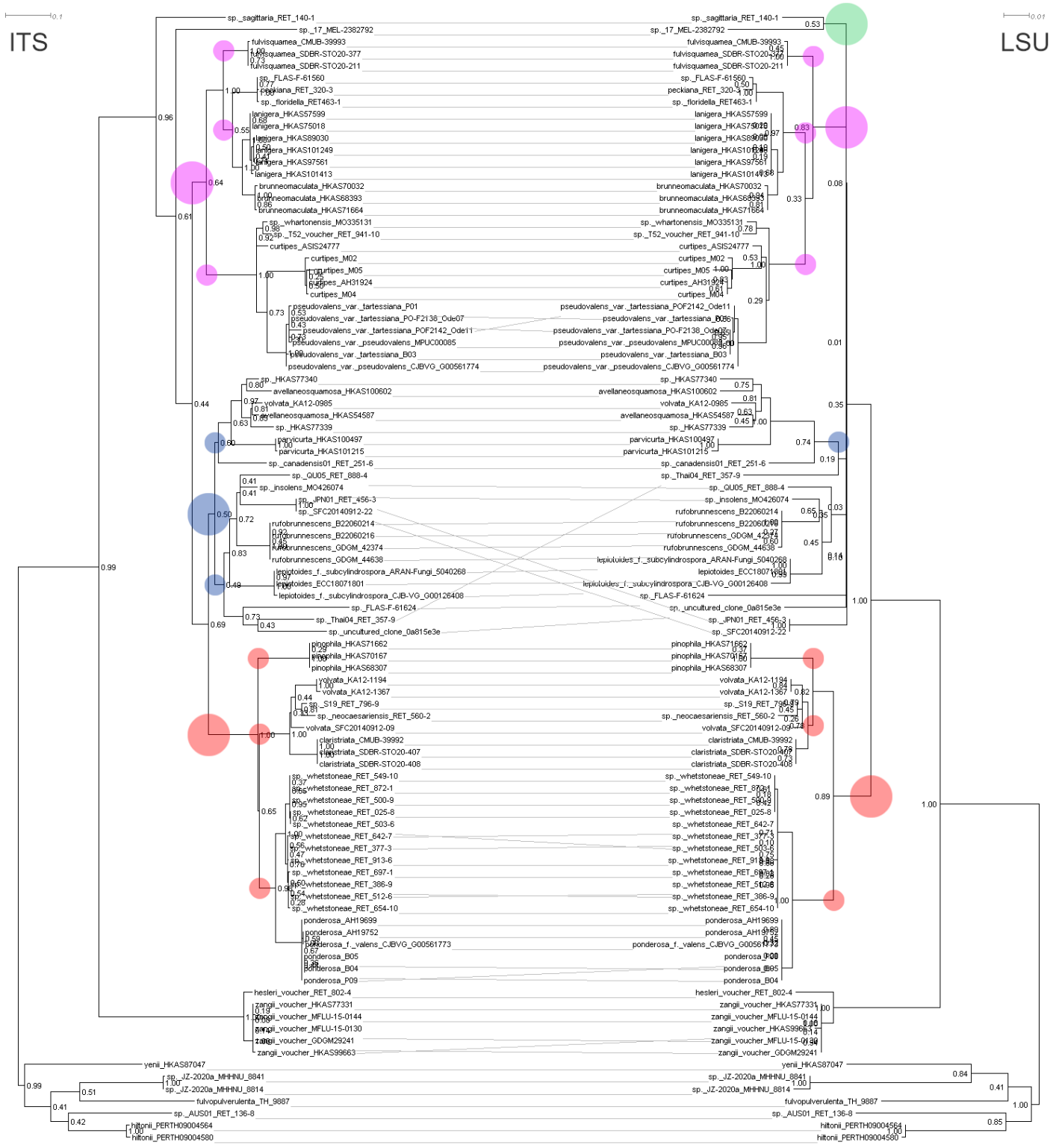
90
0.10

# Fig. 2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curtipes_M02
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pseudovalens_var._tartessiana_B03
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avellaneosquamosa_HKAS100602
volvata_KA12-0985
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sp._Thai04_RET_357-9
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pinophila_HKAS70167
pinophila_HKAS68307
volvata_KA12-1194
volvata_KA12-1367
sp._S19_RET_796-9
sp._neocaesariensis_RET_560-2
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claristriata_CMUB-39992
claristriata_SDBR-STO20-407
claristriata_SDBR-STO20-408
sp._whetstoneae_RET_549-10
sp._whetstoneae_RET_872-1
sp._whetstoneae_RET_500-9
sp._whetstoneae_RET_025-8
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ponderosa_B05
ponderosa_B04
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yenii_HKAS87047
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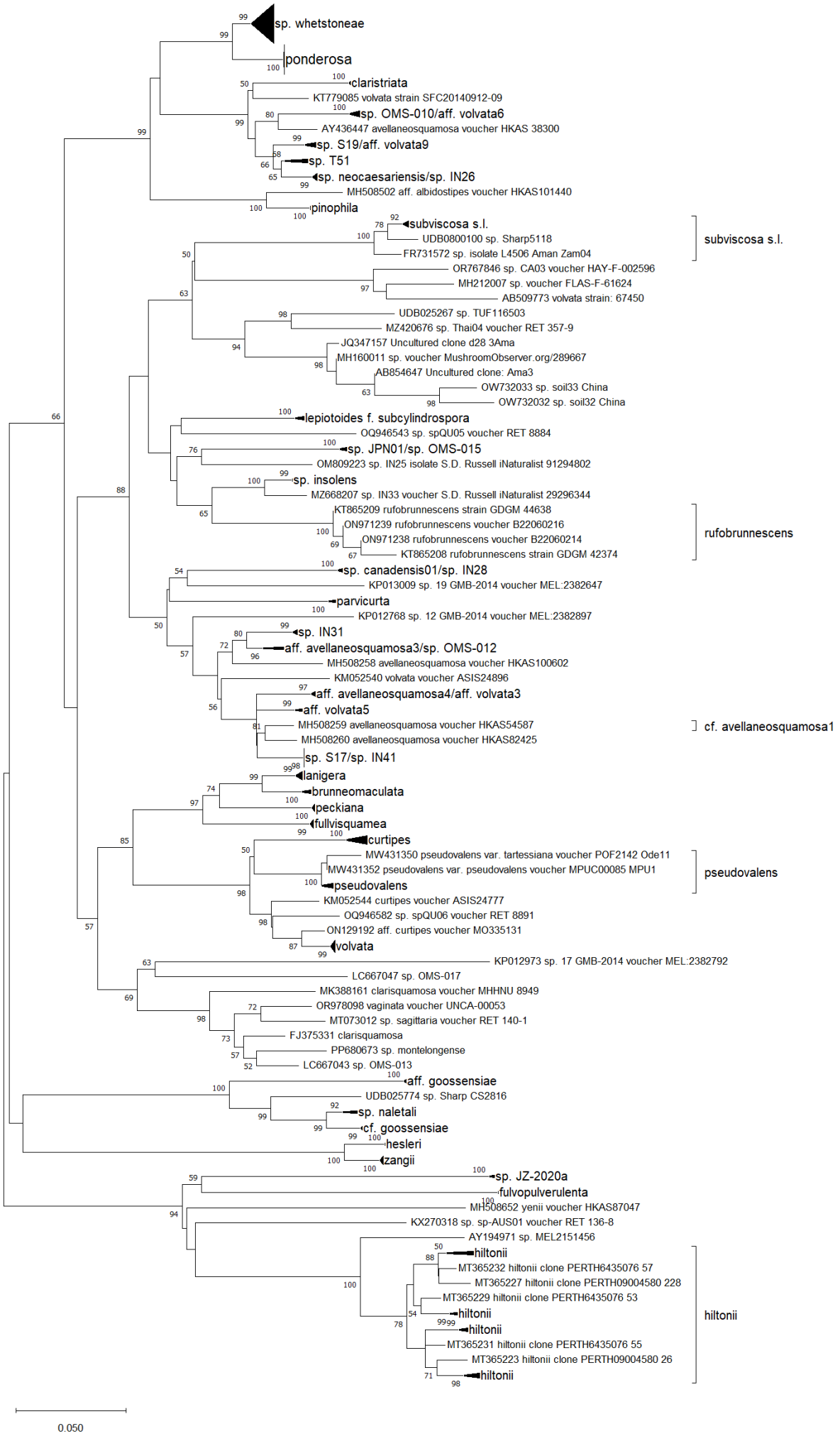
# Fig. 3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claristriata
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sp. S19/aff. volvata9
sp. T51
sp. neocaesariensis/sp. IN26
MH508502 aff. albidostipes voucher HKAS101440
pinophila
subviscosa s.l.
UDB0800100 sp. Sharp5118
FR731572 sp. isolate L4506 Aman Zam04
subviscosa s.l.
OR767846 sp. CA03 voucher HAY-F-002596
MH212007 sp. voucher FLAS-F-61624
AB509773 volvata strain: 67450
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MZ420676 sp. Thai04 voucher RET 357-9
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MH160011 sp. voucher MushroomObserver.org/289667
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OW732032 sp. soil32 China
lepiotoides f. subcylindrospora
OQ946543 sp. spQU05 voucher RET 8884
sp. JPN01/sp. OMS-015
OM809223 sp. IN25 isolate S.D. Russell iNaturalist 91294802
sp. insolens
MZ668207 sp. IN33 voucher S.D. Russell iNaturalist 29296344
KT865209 rufobrunnescens strain GDGM 44638
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rufobrunnescens
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parvicurta
KP012768 sp. 12 GMB-2014 voucher MEL:2382897
sp. IN31
aff. avellaneosquamosa3/sp. OMS-012
MH508258 avellaneosquamosa voucher HKAS100602
KM052540 volvata voucher ASIS24896
aff. avellaneosquamosa4/aff. volvata3
aff. volvata5
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cf. avellaneosquamosa1
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lanigera
brunneomaculata
peckiana
fullvisquamea
curtipes
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pseudovalens
pseudovalens
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volvata
KP012973 sp. 17 GMB-2014 voucher MEL:2382792
LC667047 sp. OMS-017
MK388161 clarisquamosa voucher MHHNU 8949
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MT073012 sp. sagittaria voucher RET 140-1
FJ375331 clarisquamosa
PP680673 sp. montelongense
LC667043 sp. OMS-013
aff. goossensiae
UDB025774 sp. Sharp CS2816
sp. naletali
cf. goossensiae
hesleri
zangii
sp. JZ-2020a
fulvopulverulenta
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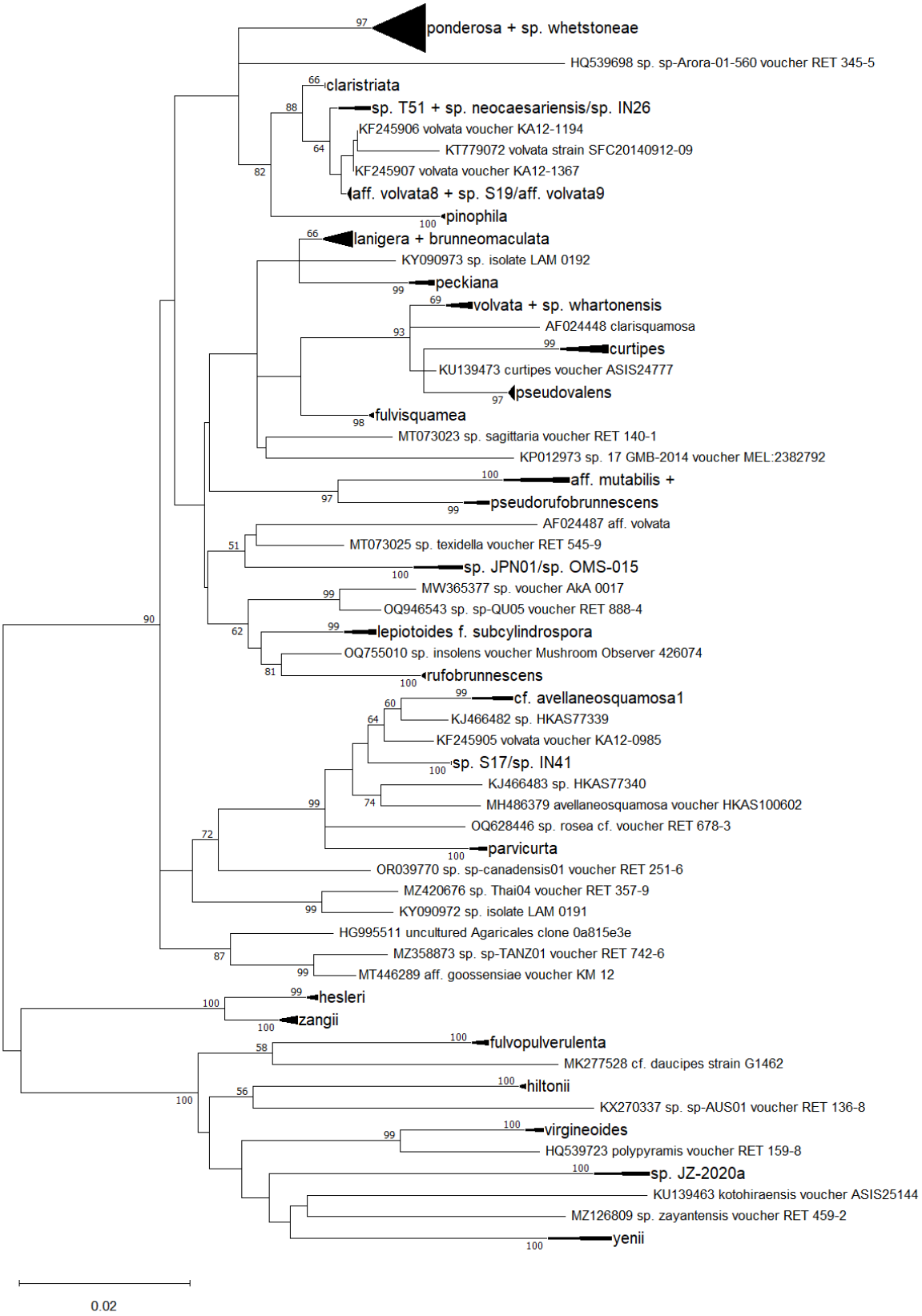
# Fig. 4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KT779072 volvata strain SFC20140912-09
KF245907 volvata voucher KA12-1367
aff. volvata8 + sp. S19/aff. volvata9
pinophila
lanigera + brunneomaculata
KY090973 sp. isolate LAM 0192
peckiana
volvata + sp. whartonensis
AF024448 clarisquamosa
curtipes
KU139473 curtipes voucher ASIS24777
pseudovalens
fulvisquamea
MT073023 sp. sagittaria voucher RET 140-1
KP012973 sp. 17 GMB-2014 voucher MEL:2382792
aff. mutabilis +
pseudorufobrunnescens
AF024487 aff. volvata
MT073025 sp. texidella voucher RET 545-9
sp. JPN01/sp. OMS-015
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rufobrunnescens
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KF245905 volvata voucher KA12-0985
sp. S17/sp. IN41
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OQ628446 sp. rosea cf. voucher RET 678-3
parvicurta
OR039770 sp. sp-canadensis01 voucher RET 251-6
MZ420676 sp. Thai04 voucher RET 357-9
KY090972 sp. isolate LAM 0191
HG995511 uncultured Agaricales clone 0a815e3e
MZ358873 sp. sp-TANZ01 voucher RET 742-6
MT446289 aff. goossensiae voucher KM 12
hesleri
zangii
fulvopulverulenta
MK277528 cf. daucipes strain G1462
hiltonii
KX270337 sp. sp-AUS01 voucher RET 136-8
virgineoides
HQ539723 polypyramis voucher RET 159-8
sp. JZ-2020a
KU139463 kotohiraensis voucher ASIS25144
MZ126809 sp. zayantensis voucher RET 459-2
yenii
0.02

Fig. 5a

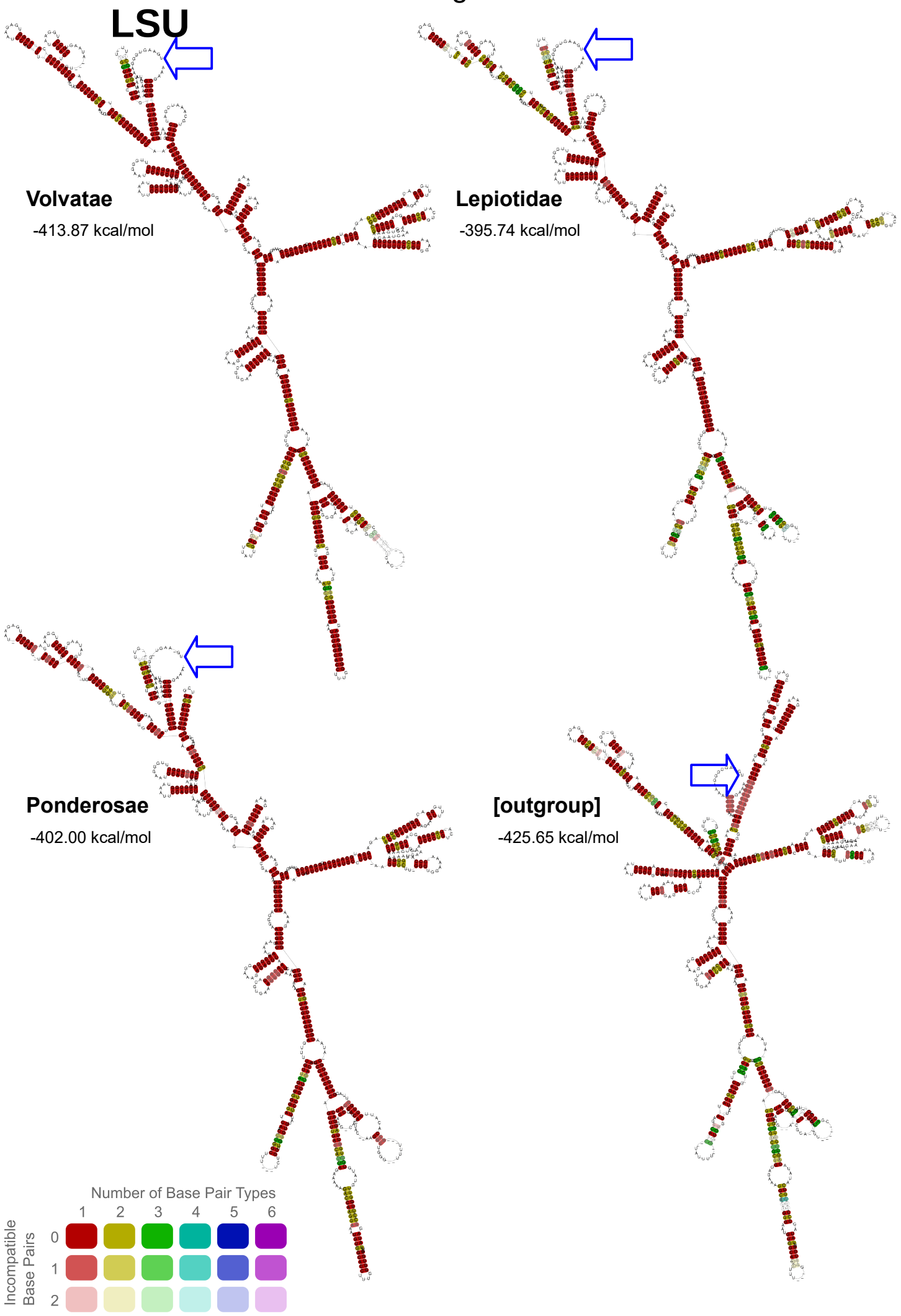
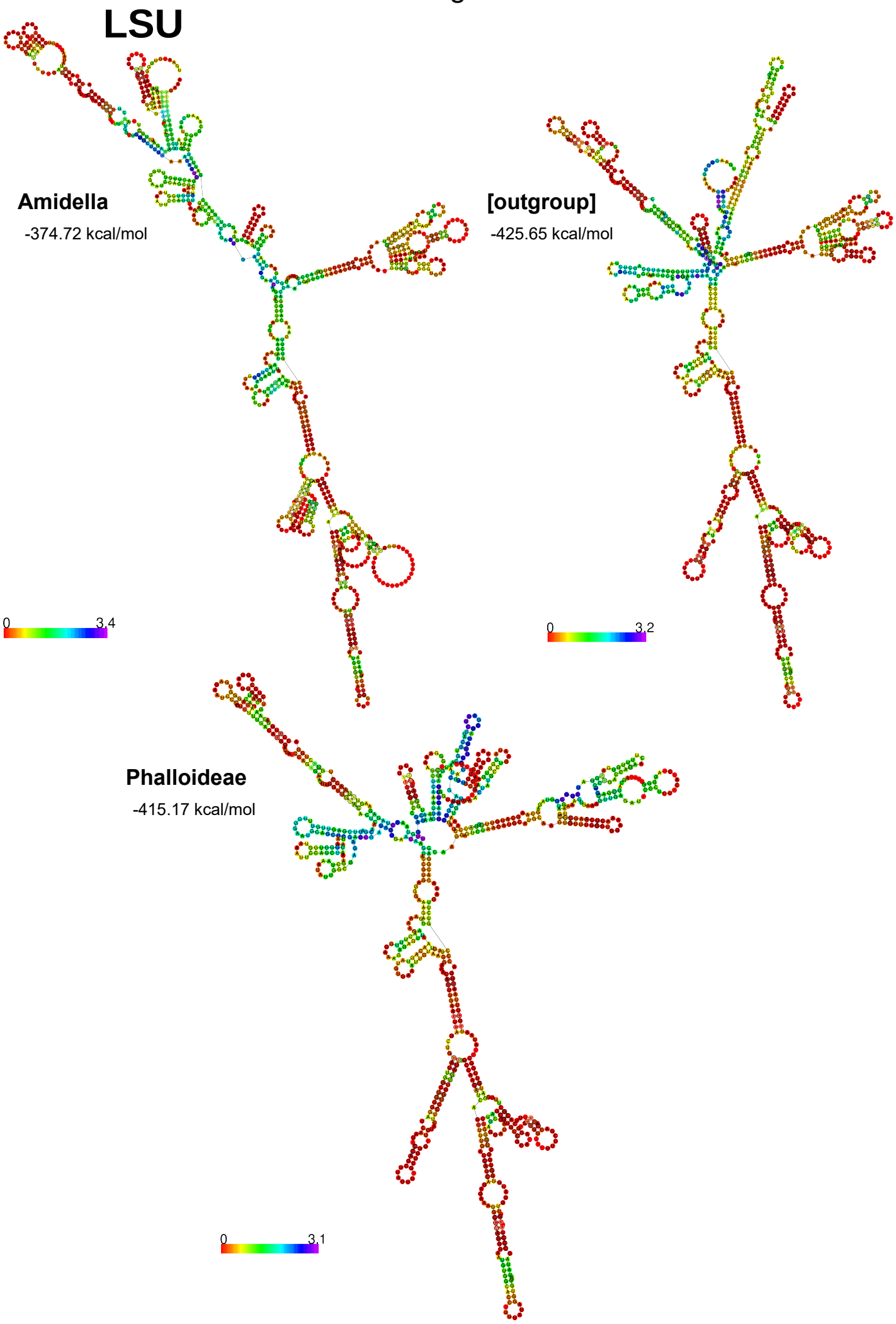

LSU
Volvatae
-413.87 kcal/mol
Lepiotidae
-395.74 kcal/mol
Ponderosae
-402.00 kcal/mol
[outgroup]
-425.65 kcal/mol
Number of Base Pair Types
1 2 3 4 5 6
Incompatible Base Pairs
0
1
2

Fig. 5b


LSU
Amidella
-374.72 kcal/mol
0
3.4
[outgroup]
-425.65 kcal/mol
0
3.2
Phalloideae
-415.17 kcal/mol
0
3.1